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☐ 1. Document ID: US 6569665 B1

L2: Entry 1 of 51 File: USPT May 27, 2003

US-PAT-NO: 6569665
DOCUMENT-IDENTIFIER: US 6569665 B1

TITLE: Calpaines, production and use thereof

DATE-ISSUED: May 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Boehm; Thomas	Vorstetten			DE
Dear; Neil T.	Heidelberg			DE

US-CL-CURRENT: 435/226; 435/183, 435/219, 435/252.3, 435/254.1, 435/325, 435/348, 435/69.1, 536/23.2, 536/23.5, 536/24.1 , 536/24.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☐ 2. Document ID: US 6562958 B1

L2: Entry 2 of 51 File: USPT May 13, 2003

US-PAT-NO: 6562958
DOCUMENT-IDENTIFIER: US 6562958 B1

TITLE: Nucleic acid and amino acid sequences relating to Acinetobacter baumannii for diagnostics and therapeutics

DATE-ISSUED: May 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Breton; Gary	Marlborough	MA		
Bush; David	Somerville	MA		

US-CL-CURRENT: 536/23.7; 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

☐ 3. Document ID: US 6559294 B1

L2: Entry 3 of 51 File: USPT May 6, 2003

US-PAT-NO: 6559294
DOCUMENT-IDENTIFIER: US 6559294 B1

TITLE: Chlamydia pneumoniae polynucleotides and uses thereof

DATE-ISSUED: May 6, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Griffais; Remy	Momtrouge			FR
Hoiseth; Susan K.	Fairport	NY		
Zagursky; Robert John	Victor	NY		
Metcalf; Benjamin J.	Rochester	NY		
Peek; Joel A.	Pittsford	NY		
Sankaran; Banumathi	Penfield	NY		
Fletcher; Leah Diane	Geneseo	NY		

US-CL-CURRENT: 536/23.1; 435/320.1, 435/69.1, 435/70.1, 536/24.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw. Desc | Image

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☐ 4. Document ID: US 6551795 B1

L2: Entry 4 of 51 File: USPT Apr 22, 2003

US-PAT-NO: 6551795
DOCUMENT-IDENTIFIER: US 6551795 B1

TITLE: Nucleic acid and amino acid sequences relating to pseudomonas aeruginosa for diagnostics and therapeutics

DATE-ISSUED: April 22, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rubenfield; Marc J.	Framingham	MA		
Nolling; Jork	Ouincy	MA		
Deloughery; Craig	Medford	MA		
Bush; David	Somerville	MA		

US-CL-CURRENT: 435/69.1; 435/253.3, 435/320.1, 435/325, 435/6, 536/23.1, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 5. Document ID: US 6548287 B1

L2: Entry 5 of 51 File: USPT Apr 15, 2003

US-PAT-NO: 6548287
DOCUMENT-IDENTIFIER: US 6548287 B1

TITLE: Non-pyrogenic bacterial strains and use of the same

DATE-ISSUED: April 15, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Powell; Robert J.	Baltimore	MD		
Hone; David M.	Ellicott City	MD		

US-CL-CURRENT: 435/243; 424/234.1, 424/241.1, 424/245.1, 424/249.1, 424/253.1, 424/258.1, 424/260.1, 424/261.1, 435/170 , 435/252.3, 435/69.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 6. Document ID: US 6537558 B2

L2: Entry 6 of 51 File: USPT Mar 25, 2003

US-PAT-NO: 6537558
DOCUMENT-IDENTIFIER: US 6537558 B2

TITLE: Methods of producing and using virulence attenuated poxR mutant bacteria

DATE-ISSUED: March 25, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kaniga; Kone	St. Louis	MO		

US-CL-CURRENT: 424/234.1; 424/235.1, 424/241.1, 424/258.1, 435/243, 435/252.3

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 7. Document ID: US 6528289 B1

L2: Entry 7 of 51 File: USPT Mar 4, 2003

US-PAT-NO: 6528289
DOCUMENT-IDENTIFIER: US 6528289 B1

TITLE: Nucleotide sequence of the Haemophilus influenzae Rd genome, fragments thereof, and uses thereof

DATE-ISSUED: March 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fleischmann; Robert D.	Gaithersburg	MD		
Adams; Mark D.	N. Potomac	MD		
White; Owen	Gaithersburg	MD		
Smith; Hamilton O.	Towson	MD		
Venter; J. Craig	Potomac	MD		

US-CL-CURRENT: 435/91.41; 435/252.3, 435/320.1, 435/6, 536/23.1, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 8. Document ID: US 6506581 B1

L2: Entry 8 of 51 File: USPT Jan 14, 2003

US-PAT-NO: 6506581
DOCUMENT-IDENTIFIER: US 6506581 B1

TITLE: Nucleotide sequence of the Haemophilus influenzae Rd genome, fragments thereof, and uses thereof

DATE-ISSUED: January 14, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fleischmann; Robert D.	Gaithersburg	MD		
Adams; Mark D.	N. Potomac	MD		
White; Owen	Gaithersburg	MD		
Smith; Hamilton O.	Towson	MD		
Venter; J. Craig	Potomac	MD		

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1, 435/69.3, 435/91.41, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 9. Document ID: US 6489136 B1

L2: Entry 9 of 51 File: USPT Dec 3, 2002

US-PAT-NO: 6489136
DOCUMENT-IDENTIFIER: US 6489136 B1

TITLE: Cell proliferation related genes

DATE-ISSUED: December 3, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Zervos; Antonis S.	Woburn	MA		

US-CL-CURRENT: 435/69.1; 435/252.3, 435/252.33, 435/254.11, 435/320.1, 435/325,

435/410, 536/23.5, 536/24.3, 536/24.31

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

☐ 10. Document ID: US 6475793 B1

L2: Entry 10 of 51 File: USPT Nov 5, 2002

US-PAT-NO: 6475793
DOCUMENT-IDENTIFIER: US 6475793 B1

TITLE: Genomic sequence of Rhizobium sp. NGR 234 symbiotic plasmid

DATE-ISSUED: November 5, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Rosenthal; Andre	Berlin			DE
Freiberg; Christoph Bernward	Wuppertal			DE
Perret; Xavier Philippe	Geneva			CH
Broughton; William John	Geneva			CH

US-CL-CURRENT: 435/419; 435/183, 435/252.3, 435/320.1, 435/410, 435/69.1, 536/23.2, 800/278, 800/295

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 11. Document ID: US 6461854 B1

L2: Entry 11 of 51 File: USPT Oct 8, 2002

US-PAT-NO: 6461854
DOCUMENT-IDENTIFIER: US 6461854 B1

TITLE: Methods of screening compounds useful for prevention of infection or pathogenicity

DATE-ISSUED: October 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Ausubel; Frederick M.	Newton	MA		
Rahme; Laurence G.	Brookline	MA		
Tan; Man-Wah	Somerville	MA		
Ruvkun; Gary B.	Cambridge	MA		

US-CL-CURRENT: 435/252.3; 424/234.1, 424/9.1, 435/243, 435/252.34, 435/4, 435/410, 435/42

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

☐ 12. Document ID: US 6423312 B1

L2: Entry 12 of 51 File: USPT Jul 23, 2002

US-PAT-NO: 6423312
DOCUMENT-IDENTIFIER: US 6423312 B1

TITLE: Compositions including glycosaminoglycans degrading enzymes and use of same against surface protected bacteria

DATE-ISSUED: July 23, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yacoby-Zeevi; Oron	Meitar			IL

US-CL-CURRENT: 424/94.5; 424/94.1, 424/94.61, 424/94.62, 435/183, 435/200, 435/209, 435/252.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

☐ 13. Document ID: US 6413768 B1

L2: Entry 13 of 51 File: USPT Jul 2, 2002

US-PAT-NO: 6413768
DOCUMENT-IDENTIFIER: US 6413768 B1

TITLE: Expression plasmids

DATE-ISSUED: July 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Galen; James E.	Owings Mills	MD		

US-CL-CURRENT: 435/320.1; 530/300, 530/350, 530/403, 536/24.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 14. Document ID: US 6406887 B1

L2: Entry 14 of 51 File: USPT Jun 18, 2002

US-PAT-NO: 6406887
DOCUMENT-IDENTIFIER: US 6406887 B1

TITLE: Compositions for diagnosing Rochalimaea henselae and Rochalmaea quintana infection

DATE-ISSUED: June 18, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Anderson; Burt E.	Valrico	FL		
Regnery; Russell L.	Tucker	GA		

US-CL-CURRENT: 435/69.3; 424/185.1, 424/190.1, 424/192.1, 424/234.1, 435/6, 435/69.1, 435/7.32, 435/822, 530/350, 530/806, 530/825

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 15. Document ID: US 6391313 B1

L2: Entry 15 of 51 File: USPT May 21, 2002

US-PAT-NO: 6391313
DOCUMENT-IDENTIFIER: US 6391313 B1

TITLE: Multi-component vaccine to protect against disease caused by Haemophilus influenzae and Moraxella catarrhalis

DATE-ISSUED: May 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Loosmore; Sheena M.	Aurora			CA
Yang; Yan-Ping	Willowdale			CA
Klein; Michel H.	Willowdale			CA
Sasaki; Ken	Willowdale			CA

US-CL-CURRENT: 424/203.1; 424/193.1, 424/197.11, 424/234.1, 424/251.1, 424/256.1, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 16. Document ID: US 6355469 B1

L2: Entry 16 of 51 File: USPT Mar 12, 2002

US-PAT-NO: 6355469
DOCUMENT-IDENTIFIER: US 6355469 B1

TITLE: Nucleic acid encoding M. tuberculosis algu protein

DATE-ISSUED: March 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lam; Kelvin T.	Belmont	MA		

US-CL-CURRENT: 435/252.3; 435/183, 435/194, 435/254.11, 435/320.1, 435/325, 435/348, 435/419, 435/6, 530/350, 536/23.1, 536/23.2, 536/23.7

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw. Desc | Image

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☐ 17. Document ID: US 6355450 B1

L2: Entry 17 of 51 File: USPT Mar 12, 2002

US-PAT-NO: 6355450
DOCUMENT-IDENTIFIER: US 6355450 B1

TITLE: Computer readable genomic sequence of Haemophilus influenzae Rd, fragments thereof, and uses thereof

DATE-ISSUED: March 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Fleischmann; Robert D.	Gaithersburg	MD		
Adams; Mark D.	N. Potomac	MD		
White; Owen	Gaithersburg	MD		
Smith; Hamilton O.	Towson	MD		
Venter; J. Craig	Potomac	MD		

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1, 435/851, 536/23.1, 536/23.7, 536/24.32, 536/24.33

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 18. Document ID: US 6342232 B1

L2: Entry 18 of 51 File: USPT Jan 29, 2002

US-PAT-NO: 6342232
DOCUMENT-IDENTIFIER: US 6342232 B1

TITLE: Multi-component vaccine comprising at least three antigens to protect against disease cased by Haemophilus influenzae

DATE-ISSUED: January 29, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Loosmore; Sheena M.	Aurora			CA
Yang; Yan-Ping	Willowdale			CA
Klein; Michel H.	Willowdale			CA

US-CL-CURRENT: 424/256.1; 424/193.1, 424/200.1, 424/201.1, 424/202.1, 424/203.1, 424/282.1, 435/69.1, 530/350

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC | Draw Desc | Image

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☐ 19. Document ID: US 6335170 B1

L2: Entry 19 of 51 File: USPT Jan 1, 2002

US-PAT-NO: 6335170
DOCUMENT-IDENTIFIER: US 6335170 B1

TITLE: Gene expression in bladder tumors

DATE-ISSUED: January 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Orntoft; Torben F.	DK 8230 Aabyhoj			DK

US-CL-CURRENT: 435/6; 435/91.1, 435/91.2, 536/23.1, 536/24.3, 536/24.31, 536/24.33

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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☐ 20. Document ID: US 6316609 B1

L2: Entry 20 of 51 File: USPT Nov 13, 2001

US-PAT-NO: 6316609
DOCUMENT-IDENTIFIER: US 6316609 B1

TITLE: Nucleotide sequence of Escherichia coli pathogenicity islands

DATE-ISSUED: November 13, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dillon; Patrick J.	Gaithersburg	MD		
Choi; Gil H.	Rockville	MD		
Welch; Rodney A.	Madison	WI		

US-CL-CURRENT: 536/23.1; 435/252.3, 435/252.33, 435/320.1, 435/325, 536/24.3, 536/24.32

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | KWIC
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L2: Entry 41 of 51 File: USPT Sep 8, 1998

DOCUMENT-IDENTIFIER: US 5804194 A
TITLE: Vaccines containing a salmonella bacteria attenuated by mutation of the htra gene

Brief Summary Text (4):
The genes encoding the family of heat shock proteins are transcribed by RNA polymerase co-operating with the .sigma..sup.32 factor, the product of the rpoH gene (reviewed by Neidhardt, F. C. and van Bogelen, R. A, 1987. In Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology, Neidhardt, F. C. et al eds. pp. 1334-1345, American Society for Microbiology, Washington, D.C.). Recently, Lipinska et al (Nucleic.Acids.Res. 1988 21, 10053-10067) have described a heat shock protein in E.coli, referred to as HtrA, that appears to be .sigma..sup.32 -independent. Examination of the promoter region of the htrA gene shows DNA sequence homology with the P.3 promoter of the rpoH gene; a promoter known to be recognised by .sigma..sup.E (.sigma..sup.24) factor. This similarity suggests that the htrA promoter may also be recognised by the RNA polymerase-.sigma..sup.E (.sigma..sup.24)holoenzyme.

Brief Summary Text (5):
Phenotypically, in E.coli, a mutation in the htrA locus abolishes the ability of bacterium to survive at temperatures above 42.degree. C. (Lipinska et al, 1989, J.Bacteriol, 171, 1574-1584). The gene maps at 4 min on the E.coli chromosome and encodes a protein with a relative molecular mass (Mr) of 51,163. This protein precursor undergoes N-terminal processing involving the removal of a signal peptide sequence (Lipinska et al, 1988, Nucleic.Acids.Res. 21, 10053-10067), to yield the mature form of the polypeptide upon secretion through the inner membrane of the bacterium. Independently, the htrA gene has been identified as degP by Strauch, K. L. and Beckwith, J. 1988 (Proc.Natl.Acad.Sci. USA 85, 1576-1580) who were examining E.coli mutants with decreased protease activity, degP mutants were isolated by TnphoA mutagenesis (Manoil, C. & Beckwith, J. 1985, Proc.Natl.Acad.Sci. USA 82, 8129-8133) and were recognised by the increased stability of a hybrid Tsr-phoA (Tsr-AP2) recombinant protein in a degP background (Strauch, K. L. and Beckwith, J. 1988. Proc.Natl. Acad.Sci. USA 85, 1576-1680). In E.coli the genes identified as degP and htrA appear to be identical and encode a protein that is a member of the `stress-response` family.

Brief Summary Text (11):
Preferably a heat shock protein is the one encoded by the htrA gene as set out in FIG. 1. (SEQ ID No: 1) (also characterised as degP). Other proteins are encoded by genes known to be involved in the stress response such as grpE, groEL, (moPA), dnaK, groES, lon and dnaJ. There are many other proteins encoded by genes which are known to be induced in response to environmental stress (Ronson et al, Cell 49, 579-581). Amongst these the following can be mentioned: the ntrB/ntrC system of E.coli, which is induced in response to nitrogen deprivation and positively regulates glnA and nifLA (Buck et al., Nature 320, 374-378, 1986; Hirschman et al., Proc.Natl.Acad.Sci. USA, 82, 7525, 1985; Nixon et al., Proc.Natl.Acad.Sci. USA 83, 7850-7854, 1986, Reitzer and Magansanik, Cell, 45, 785, 1986); the phoR/phoB system of E.coli which is induced in response to phosphate deprivation (Makino et al., J.Mol.Biol. 192, 549-556, 1986b); the cpxA/sfrA system of E.coli which is induced in response to dyes and other toxic compounds (Albin et al., J.Biol.Chem. 261 4698, 1986; Drury et al., J.Biol.Chem. 260, 4236-4272, 1985). An analogous system in Rhizobium is dctB/dctD, which is responsive to 4C-discarboxylic acids (Ronson et al., J.Bacteriol. 169, 2424

and Cell 49, 579-581, 1987). A virulence system of this type has been described in Agrobacterium. This is the virA/virG system, which is induced in response to plant exudates (le Roux et al., EMBO J. 6, 849-856, 1987; Stachel and Zambryski., Am.J.Vet.Res. 45, 59-66, 1986; Winans et al., Proc.Natl. Acad.Sci. USA, 83, 8278, 1986). Similarly the bvgC-bvgA system in Bordetella pertussis (previously known as vir) regulates the production of virulence determinants in response to fluctuations in Mg2+ and nicotinic acid levels (Arico et al, 1989, Proc.Natl.Acad.Sci. USA 86, 6671-6675).

Brief Summary Text (18):
The attenuated microorganism of the present invention is optionally capable of expressing a heterologous antigen. This expression is likely to be more favourable in htrA mutants because of the increased stability of recombinant antigens associated with the degP phenotype. Such antigens may be viral, bacterial, protozoal or of higher parasitic microorganisms. Such microorganisms may then form the basis of a bi- or multi-valent vaccine. Examples of useful antigens include E.coli heat labile toxin B subunit (LT-B), E.coli K88 antigens, FMDV (Foot and Mouth) peptides, Influenza viral proteins, P.69 protein from B.pertussis. Other antigens which could be usefully expressed would be those from Chlamydia, flukes, mycoplasma, roundworms, tapeworms, rabies virus and rotavirus.

Drawing Description Text (2):
FIGS. 1A and B. DNA sequence of the htrA gene and the amino acid sequence of the protein it encodes (SEQ ID NO:1 corresponds to the DNA sequence of FIG. 1, SEQ ID NO:2 corresponds to the lower of the two amino acid sequences in FIG. 1 and SEQ ID NO:3 corresponds to the upper of the amino acid sequences in FIG. 1).

Drawing Description Text (4):
FIG. 3. In vivo kinetics of S.typhimurium strains harbouring a mutation in htrA (BRD726) and htrA aro mutations (BRD807).

Detailed Description Text (2):
Identification of the htrA gene in Salmonella typhimurium and generation of an htrA mutant.

Detailed Description Text (3):
TnphoA mutagenesis was used in the mouse virulent Salmonella typhimurium strain C5 (Miller et al, 1989, Infect.Immunol, 57, 2758-2763). Mutants were selected likely to harbour lesions in genes that have a signal peptide sequence, i.e. proteins likely to be targeted through a bacterial membrane. Isolation of the DNA flanking the TnphoA insertion identifies the gene that has been insertionally activated. This gene was isolated and its DNA sequence was determined by standard methods (see FIG. 1. SEQ ID No: 1) (Maniatis et al., 1982, In Molecular Cloning: A laboratory manual. Cold Spring Harbour Laboratory, Cold Spring Harbour, N.Y.; Sanger et al., 1977, Proc.Natl.Acad.Sci. USA 74, 5463-5467). Comparison of the translated protein sequence with sequences held in the EMBL Database showed surprisingly that it shared 88% homology with the sequence of the htrA product from E.coli (FIG. 1. SEQ ID No: 1).

Detailed Description Text (5):
Identification of htrA in S.typhimurium as a gene involved in the stress-response

Detailed Description Text (6):
E.Coli mutants harbouring lesions in the htrA gene are unable to grow at temperatures above 42.degree. C. The S.typhimurium htrA mutant, 046, was tested for growth at elevated temperatures and was found to grow as well as the parent strain C5. However, when tested for sensitivity to oxygen radicals, the mutant 046 showed decreased resistance as compared with the parent C5 strain clearly indicating that the gene is responsible (at least in part) for this aspect of the stress response (see FIG. 2).

Detailed Description Text (15):
Construction of a defined S.typhimurium SL1344 htrA mutant

Detailed Description Text (16):
Sequence data facilitated the identification of suitable restriction endonuclease sites that could be used to introduce a deletion into the htrA gene. A 1.2 Kb

deletion was introduced by digesting with EcoRV and religating. A drug resistant marker was also introduced into the gene (Kanamycin cassette, Pharmacia) by standard techniques to enable selection for the presence of the deleted gene. The plasmid harbouring the deleted htrA gene was introduced into a polA strain S.typhimurium (BRD207) in which the plasmid cannot replicate. The only way that kanamycin resistance can be maintained in the host is if there has been a recombination event between the S.typhimurium sequences on the vector and the homologous regions on the chromosome. Loss of ampicillin resistance while maintaining kanamycin resistance indicates a second homologous recombination event resulting in the replacement of the intact htrA gene with the deleted one. Colonies resistant to kanamycin were isolated and checked for ampicillin resistance. One colony that was kanamycin resistant and ampicillin sensitive was selected for further study and was designated BRD698 (deposited at PHLS, NCTC, 61 Colindale Avenue, London NW9 5HT under Accession No. NCTC 12457 on Mar. 22, 1991 in accordance with the terms of the Budapest Treaty).

Detailed Description Text (19):
Construction of an S.typhimurium SL1344 aroA htrA double mutant

Detailed Description Text (20):
The P22 lysate prepared on BRD698 was used to introduce the htrA deletion into an S.typhimurium SL1344 strain already harbouring a deletion in aroA. The method for introducing an aroA deletion has already been described by Dougan et al, J.Infect.Dis. 158, 1329-1335, 1988. One strain that was found to have deletions in both aroA and htrA was selected for further study and was designated BRD807, (deposited at PHLS under Accession No. NCTC 12459 on Mar. 22, 1991 in accordance with the terms of the Budapest Treaty).

Detailed Description Text (22):
Comparison of the attenuation of SL1344 htrA (BRD726) and SL1344 htrA and aroA (BRD807) with the virulent parent strain SL1344

CLAIMS:

1. A vaccine comprising a prophylactically effective amount of a Salmonella bacterium attenuated by a non-reverting mutation in the htrA gene and a pharmaceutically acceptable carrier.

10. The method of prophylactic treatment of a host for an infection caused by Salmonella which comprises administering to said host a prophylactically effective dose of a Salmonella bacterium attenuated by a non-reverting mutation in the htrA gene.

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L2: Entry 34 of 51 File: USPT Nov 9, 1999

DOCUMENT-IDENTIFIER: US 5980907 A
TITLE: Vaccines containing bacteria attenuated by mutation of the htrA gene

Brief Summary Text (4):
The genes encoding the family of heat shock proteins are transcribed by RNA polymerase co-operating with the .sigma..sup.32 factor, the product of the rpoH gene (reviewed by Neidhardt, F. C. and van Bogelen, R. A., 1987. In Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology, Neidhardt, F. C. et al eds. pp. 1334-1345, American Society for Microbiology, Washington, D.C.). Recently, Lipinska et al (Nucleic.Acids.Res. 1988 21, 10053-10067) have described a heat shock protein in E.coli, referred to as HtrA, that appears to be .sigma..sup.32 -independent. Examination of the promoter region of the htrA gene shows DNA sequence homology with the P.3 promoter of the rpoH gene; a promoter known to be recognised by .sigma..sup.E (.sigma..sup.24) factor. This similarity suggests that the htrA promoter may also be recognised by the RNA polymerase-.sigma..sup.E (.sigma..sup.24)holoenzyme.

Brief Summary Text (5):
Phenotypically, in E.coli, a mutation in the htrA locus abolishes the ability of bacterium to survive at temperatures above 42.degree. C. (Lipinska et al, 1989, J.Bacteriol, 171, 1574-1584). The gene maps at 4 min on the E.coli chromosome and encodes a protein with a relative molecular mass (Mr) of 51,163. This protein precursor undergoes N-terminal processing involving the removal of a signal peptide sequence (Lipinska et al, 1988, Nucleic.Acids.Res. 21, 10053-10067), to yield the mature form of the polypeptide upon secretion through the inner membrane of the bacterium. Independently, the htrA gene has been identified as degP by Strauch, K. L. and Beckwith, J. 1988 (Proc.Natl.Acad.Sci. U.S.A. 85, 1576-1580) who were examining E.coli mutants with decreased protease activity, degP mutants were isolated by TnphoA mutagenesis (Manoil, C. & Beckwith, J. 1985, Proc.Natl.Acad.Sci. U.S.A. 82, 8129-8133) and were recognised by the increased stability of a hybrid Tsr-phoA (Tsr-AP2) recombinant protein in a degP background (Strauch, K. L. and Beckwith, J. 1988. Proc.Natl. Acad.Sci. U.S.A. 85, 1576-1680). In E.coli the genes identified as degP and htrA appear to be identical and encode a protein that is a member of the `stress-response` family.

Brief Summary Text (11):
Preferably a heat shock protein is the one encoded by the htrA gene as set out in FIG. 1. (SEQ ID No: 1) (also characterised as degP). Other proteins are encoded by genes known to be involved in the stress response such as grpE, groEL, (moPA), dnaK, groES, Ion and dnaJ. There are many other proteins encoded by genes which are known to be induced in response to environmental stress (Ronson et al, Cell 49, 579-581). Amongst these the following can be mentioned: the ntrB/ntrC system of E.coli, which is induced in response to nitrogen deprivation and positively regulates glnA and nifLA (Buck et al., Nature 320, 374-378, 1986; Hirschman et al., Proc.Natl.Acad.Sci. U.S.A., 82, 7525, 1985; Nixon et al., Proc.Natl.Acad.Sci. U.S.A. 83, 7850-7854, 1986, Reitzer and Magansanik, Cell, 45, 785, 1986); the phoR/phoB system of E.coli which is induced in response to phosphate deprivation (Makino et al., J.Mol.Biol. 192, 549-556, 1986b); the cpxA/sfrA system of E.coli which is induced in response to dyes and other toxic compounds (Albin et al., J.Biol.Chem. 261 4698, 1986; Drury et al., J.Biol.Chem. 260, 4236-4272, 1985). An analogous system in Rhizobium is dctB/dctD, which is responsive to 4C-discarboxylic acids (Ronson et al., J.Bacteriol. 169, 2424 and Cell 49, 579-581, 1987). A virulence system of this type has been described in

Agrobacterium. This is the virA/virG system, which is induced in response to plant exudates (le Roux et al., EMBO J. 6, 849-856, 1987; Stachel and Zambryski., Am.J.Vet.Res. 45, 59-66, 1986; Winans et al., Proc.Natl. Acad.Sci. U.S.A., 83, 8278, 1986). Similarly the bvgC-bvgA system in Bordetella pertussis (previously known as vir) regulates the production of virulence determinants in response to fluctuations in Mg2+ and nicotinic acid levels (Arico et al, 1989, Proc.Natl.Acad.Sci. U.S.A. 86, 6671-6675).

Brief Summary Text (18):
The attenuated microorganism of the present invention is optionally capable of expressing a heterologous antigen. This expression is likely to be more favourable in htrA mutants because of the increased stability of recombinant antigens associated with the degP phenotype. Such antigens may be viral, bacterial, protozoal or of higher parasitic microorganisms. Such microorganisms may then form the basis of a bi- or multi-valent vaccine. Examples of useful antigens include E.coli heat labile toxin B subunit (LT-B), E.coli K88 antigens, FMDV (Foot and Mouth) peptides, Influenza viral proteins, P.69 protein from B.pertussis. Other antigens which could be usefully expressed would be those from Chlamydia, flukes, mycoplasma, roundworms, tapeworms, rabies virus and rotavirus.

Drawing Description Text (2):
FIGS. 1A and 1B. DNA sequence of the htrA gene and the amino acid sequence of the protein it encodes (SEQ ID NO:1 corresponds to the DNA sequence of FIG. 1, SEQ ID NO:2 corresponds to the lower of the two amino acid sequences in FIG. 1 and SEQ ID NO:3 corresponds to the upper of the amino acid sequences in FIG. 1).

Drawing Description Text (3):
FIG. 2. Sensitivity of S.typhimurium htrA mutant 046 to temperatures above 42.degree. C. and oxygen radicals

Drawing Description Text (4):
FIG. 3. In vivo kinetics of S.typhimurium strains harbouring a mutation in htrA (BRD726) and htrA aro mutations (BRD807).

Detailed Description Text (2):
Identification of the htrA gene in Salmonella typhimurium and generation of an htrA mutant.

Detailed Description Text (3):
TnphoA mutagenesis was used in the mouse virulent Salmonella typhimurium strain C5 (Miller et al, 1989, Infect.Immunol, 57, 2758-2763). Mutants were selected likely to harbour lesions in genes that have a signal peptide sequence, i.e. proteins likely to be targeted through a bacterial membrane. Isolation of the DNA flanking the TnphoA insertion identifies the gene that has been insertionally activated. This gene was isolated and its DNA sequence was determined by standard methods (see FIG. 1. SEQ ID No: 1) (Maniatis et al., 1982, In Molecular Cloning: A laboratory manual. Cold Spring Harbour Laboratory, Cold Spring Harbour, N.Y.; Sanger et al., 1977, Proc.Natl.Acad.Sci. U.S.A. 74, 5463-5467). Comparison of the translated protein sequence with sequences held in the EMBL Database showed surprisingly that it shared 88% homology with the sequence of the htrA product from E.coli (FIG.1. SEQ ID No: 1).

Detailed Description Text (5):
Identification of htrA in S.typhimurium as a gene involved in the stress-response

Detailed Description Text (6):
E.Coli mutants harbouring lesions in the htrA gene are unable to grow at temperatures above 42.degree. C. The S.typhimurium htrA mutant, 046, was tested for growth at elevated temperatures and was found to grow as well as the present strain C5. However, when tested for sensitivity to oxygen radicals, the mutant 046 showed decreased resistance as compared with the parent C5 strain clearly indicating that the gene is responsible (at least in part) for this aspect of the stress response (see FIG. 2).

Detailed Description Text (15):

Construction of a defined S.typhimurium SL1344 htrA mutant

Detailed Description Text (16):
Sequence data facilitated the identification of suitable restriction endonuclease sites that could be used to introduce a deletion into the htrA gene. A 1.2 Kb deletion was introduced by digesting with EcoRV and religating. A drug resistant marker was also introduced into the gene (Kanamycin cassette, Pharmacia) by standard techniques to enable selection for the presence of the deleted gene. The plasmid harbouring the deleted htrA gene was introduced into a polA strain S.typhimurium (BRD207) in which the plasmid cannot replicate. The only way that kanamycin resistance can be maintained in the host is if there has been a recombination event between the S.typhimurium sequences on the vector and the homologous regions on the chromosome. Loss of ampicillin resistance while maintaining kanamycin resistance indicates a second homologous recombination event resulting in the replacement of the intact htrA gene with the deleted one. Colonies resistant to kanamycin were isolated and checked for ampicillin resistance. One colony that was kanamycin resistant and ampicillin sensitive was selected for further study and was designated BRD698 (deposited at PHLS, NCTC, 61 Colindale Avenue, London NW9 5HT under Accession No. NCTC 12457 on Mar. 22, 1991 in accordance with the terms of the Budapest Treaty). A P22 lysate was prepared on this strain by standard techniques (Dougan et al, J.Infect.Dis. 158, 1329-1335, 1988) and used to infect SL1344. Kanamycin resistant colonies were isolated and checked for the presence of the deletion by Southern hybridisation. One strain, designated BRD726 (deposited at PHLS under Accession No. NCTC 12458 on Mar. 22, 1991 in accordance with the terms of the Budapest Treaty) was selected for further study.

Detailed Description Text (18):
Construction of an S.typhimurium SL1344 aroA htrA double mutant

Detailed Description Text (19):
The P22 lysate prepared on BRD698 was used to introduce the htrA deletion into an S.typhimurium SL1344 strain already harbouring a deletion in aroA. The method for introducing an aroA deletion has already been described by Dougan et al, J.Infect.Dis. 158, 1329-1335, 1988. One strain that was found to have deletions in both aroA and htrA was selected for further study and was designated BRD807, (deposited at PHLS under Accession No. NCTC 12459 on Mar. 22, 1991 in accordance with the terms of the Budapest Treaty).

Detailed Description Text (21):
Comparison of the attenuation of SL1344 htrA (BRD726) and SL1344 htrA and aroA (BRD807) with the virulent parent strain SL1344

CLAIMS:

1. A vaccine comprising a prophylactically effective amount of a bacterium and a pharmaceutically acceptable carrier, wherein the bacterium is a Gram-negative bacterium which colonises a mucosal surface and invades and grows within a eukaryotic cell and which is attenuated by non-reverting mutations in the htrA gene and in a second gene.

2. The vaccine as claimed in claim 1, wherein the mutation in the htrA gene is a deletion mutation.

3. The vaccine as claimed in claim 1, wherein the mutation in the htrA gene is an insertion mutation.

9. A method of prophylactic treatment of a host for an infection by a Gram-negative bacterium which colonises a mucosal surface and invades and grows within a eukaryotic cell, which comprises administering to said host a prophylactically effective dose of said bacterium in a form attenuated by a non-reverting mutation in the htrA gene.

10. A method of prophylactic treatment of a host for an infection by a microorganism, which comprises administering to said host a prophylactically effective dose of a Gram-negative bacterium which colonises a mucosal surface and invades and grows within a eukaryotic cell, wherein said bacterium is attenuated by a non-reverting

mutation in the htrA gene and expresses DNA encoding a heterologous antigen from said microorganism.

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L2: Entry 21 of 51 File: USPT Oct 23, 2001

US-PAT-NO: 6306619
DOCUMENT-IDENTIFIER: US 6306619 B1

TITLE: DegP periplasmic protease a new anti-infective target and an in vitro assay for DegP protease function

DATE-ISSUED: October 23, 2001

Gram-Neg

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APPL-NO: 09/ 605858 [PALM]
DATE FILED: June 29, 2000

PARENT-CASE:
This application claims priority under 35 U.S.C. .sctn..sctn.119 and/or 365 to Ser. No. 60/140,990 filed in U.S.A. on Jun. 29, 1999; the entire content of which is hereby incorporated by reference.

INT-CL: [07] C12 Q 1/37

US-CL-ISSUED: 435/23; 435/220
US-CL-CURRENT: 435/23; 435/220

FIELD-OF-SEARCH: 435/23, 435/24, 435/7.3, 435/32, 435/220, 435/184

PRIOR-ART-DISCLOSED:

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ART-UNIT: 168

PRIMARY-EXAMINER: Mosher; Mary E.

ATTY-AGENT-FIRM: Burns, Doane, Swecker & Mathis, L.L.P.

ABSTRACT:

The DegP (HtrA) protease is a multifunctional protein essential for the removal of misfolded and aggregated proteins in the periplasm. The present invention provides an assay for inhibitors of DegP activity, comprising mixing a suspected inhibitor of DegP activity with DegP and a suitable substrate (preferably a native substrate of DegP such as PapA) and detecting changes in DegP activity. DegP has been shown to be essential for virulence in several Gram negative pathogens. Only three natural targets for DegP have been described: colicin A lysis protein (Cal), pilin subunits (K88, K99, Pap) and recently HMW1 and HMW2 from Hemophilus influenzae. In vitro, DegP has shown weak protease activity on casein and several other non-native substrates. The present inventors have identified the major pilin subunit of the Pap pilus, PapA, as a native DegP substrate and demonstrated binding and proteolysis of this substrate in vitro. Using an NH.sub.2 -terminal affinity tag the present inventors have purified PapA away from the PapD chaperone, in the presence of denaturant, to use as a proteolysis substrate. This finding will allow the identification of the DegP recognition and cleavage sites in substrate proteins, and further, allow the design of small molecule inhibitors of protease function.

8 Claims, 12 Drawing figures

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L2: Entry 22 of 51 File: USPT Feb 20, 2001

Gram-neg

DOCUMENT-IDENTIFIER: US 6190669 B1
TITLE: Attenuated mutants of salmonella which constitutively express the Vi antigen

Brief Summary Text (56):
E. CVD 908-htrA

Brief Summary Text (57):
It was found that inactivation of htrA, a gene encoding a stress protein that also functions as a serine protease, attenuates wild-type S. typhimurium in a mouse model (Chatfield et al, Microbial. Pathogenesis, 12:145-151 (1992a)). Moreover, mice immunized orally with S. typhimurium harboring a deletion mutation in htrA were protected against subsequent challenge with a lethal dose of wild-type S. typhimurium (Chatfield et al (1992a), supra). Therefore, a deletion mutation was introduced into htrA of CVD 908, resulting in strain CVD 908-htrA (Levine et al (1996), supra). A single dose of CVD908-htrA was fed to three groups of subjects who ingested 5.0.times.10.sup.7 (N=7), 5.0.times.10.sup.8 (N=8) or 5.0.times.10.sup.9 (N=7) cfu (Tacket et al (1997), supra). The CVD 908-htrA strain was as well-tolerated as the CVD 908 parent. Only one of these 22 subjects developed a low-grade fever, which was detected by routine surveillance, and was not associated with any complaints of malaise. However, 2 of the 22 subjects developed loose stools (Tacket et al (1997), supra). Similarly, the immune response was excellent: 20 of 22 individuals (91%) manifested significant rises in serum IgG O antibody, and gut-derived IgA ASCs that made antibody to O antigen were detected in 100% of the vaccinees. These immunologic responses are virtually identical to those observed in Phase 1 clinical trials in subjects immunized with comparable doses of CVD 908 (Tacket et al (1992a), supra). The one striking difference was with respect to vaccinemias. Whereas vaccinemias were detected in 12 of 18 subjects who received a 5.0.times.10.sup.7 or 5.0.times.10.sup.8 cfu dose of CVD 908, no vaccinemias were detected in any of the 22 individuals who ingested well-tolerated, highly immunogenic 5.0.times.10.sup.7-9 cfu doses of CVD 908-htrA ($p<0.001$) (Levine et al (1987b), supra; Tacket et al (1992a), supra; and Tacket et al (1997), supra). CVD 908-htrA also elicits strong cell-mediated immune responses in vaccinees comparable in strength to those recorded with CVD 908 (Tacket et al (1992a), supra; and Tacket et al (1997), supra). Based on these highly encouraging data, CVD 908-htrA has entered Phase 2 clinical trials to assess its clinical acceptability and immunogenicity in larger numbers of subjects, including children.

Brief Summary Text (63):
Two candidate S. typhi strains harboring deletions in phoP/phoQ have been constructed (Hohmann et al (1996a), supra; and Hohmann et al, Vaccine, 14:19-24 (1996b)). Strain Ty445, which also harbors a deletion in aroA, was found to be overly attenuated and only minimally immunogenic (Hohmann et al (1996b), supra). In contrast, strain Ty800, a derivative of Ty2 having deletions in only phoP,phoQ was generally well-tolerated and immunogenic when evaluated in dosage levels from 10.sup.7 to 10.sup.10 cfu in a small Phase 1 clinical trial involving 11 subjects (Hohmann et al (1996b), supra). At the highest dosage level, 1 of 3 vaccinees developed diarrhea (10 loose stools) . It is difficult to compare the immune responses of subjects who received Ty800 with those observed in recipients of CVD 908-htrA and .chi.4073, since some of the immunological assay techniques were different, and even where the same assay was used (e.g., IgA ASCs that make O antibody), considerable variation is known to occur between laboratories.

Brief Summary Text (65):
CVD 908 is well-tolerated but, is associated with vaccinemias in approximately 50% of subjects who ingest a 10.sup.7 cfu dose. Mild, but definite diarrhea, has been observed in approximately 10% of subjects who have ingested CVD 908-htrA, Ty800 or .chi.4073. The immune response to .chi.4073 was less potent than that observed after oral immunization with CVD 908, CVD 908-htrA and, apparently, Ty800 (see Table 1 above). Thus, although there exist four attenuated S. typhi strains that have completed Phase 1 clinical trials, each is associated with at least one drawback of sufficient concern that there is interest in the development of additional candidate attenuated S. typhi vaccine strains. Moreover, none of these four strains has succeeded in eliciting serum IgG anti-Vi antibody, a known protective immune response.

Brief Summary Paragraph Table (1):
TABLE 1 Mutated Vaccine Wild-type Clinical Immunological Gene Strain Parent Phenotype Phenotype galE, via EX462 Ty2 Not Immunogenic attenuated aroA, purA 541Ty CDC1080 Overly Poorly attenuated Immunogenic aroA, purA, 543Ty CDC1080 Overly Poorly Vi attenuated Immunogenic aroC, aroD CVD 908 Ty2 Attenuated Immunogenic aroC, aroD, CVD 908-htrA Ty2 Attenuated Immunogenic htrA cya, crp X3927 Ty2 Insufficiently Immunogenic attenuated cya, crp, cdt X4073 Ty2 Attenuated Immunogenic phoP/phoQ Ty800 Ty2 Attenuated Immunogenic

Drawing Description Text (10):
FIG. 9 shows the anti-S. typhi flagella antibody response in mice after intranasal immunization with strain .DELTA.guaB-A S. typhi CVD 915 or strain .DELTA.aroC, .DELTA.aroD, .DELTA.htrA S. typhi CVD 908-htrA.

Drawing Description Text (11):
FIG. 10 shows the anti-S. typhi LPS antibody response in mice after intranasal immunization with strain .DELTA.guaB-A S. typhi CVD 915 or strain .DELTA.aroC, .DELTA.aroD, .DELTA.htrA S. typhi CVD 908-htrA.

Detailed Description Text (77):
Invasion and intercellular growth were assayed using gentamicin protection assays, which were performed with slight modifications to methods previously described by Tartera et al, Infect. Immun., 61:3084-3089 (1993). Briefly, semiconfluent Henle 407 cell monolayers on 24-well plates were infected in triplicate wells with either wild-type strain Ty2; .DELTA.guaB-A CVD 915; .DELTA.aroC, .DELTA.aroD CVD 908 or .DELTA.aroC, .DELTA.aroD, .DELTA.htr CVD 908-htrA at a 50:1 ratio, for 90 min, after which extracellular organisms were killed with 100 .mu.g/ml of gentamicin for 30 min, washed (0 hrs time point), and thereafter incubated with 20 .mu.g/ml of gentamicin. At 0 hr, 4 hr and 22 hr thereafter, triplicate infected tissue culture monolayers were lyzed with sterile water and serial dilutions of that suspension cultured overnight, at 37.degree. C., on LB agar supplemented with 10 .mu.g of guanine per liter. The results are shown in Table 2 below.

Detailed Description Text (78):
As shown in Table 2 above, strain CVD 915 had an invasion capability and intracellular growth that was significantly lower than that exhibited by wild-type strain Ty2, and comparable to that exhibited by strain CVD 908-htrA. That is, as shown in Table 2 above, in two different experiments, wild-type S. typhi strain Ty2 efficiently invaded Henle 407 cells, and replicated in them over 13-fold in a 22 hr period. The .DELTA.aroC, .DELTA.aroD strain CVD 908 consistently had fewer intracellular generations, i.e., 6-fold, at 22 hr. Also as shown in Table 2 above, the mutant strain .DELTA.guaB-A CVD 915 was significantly less invasive for Henle cells than its wild-type parent or the strains CVD 908 and CVD 908-htrA, and its intracellular growth, i.e., 0-fold, was equivalent to that of the CVD 908-htrA mutant.

Detailed Description Text (93):
TT consists of a 150 kDa protein containing a 50 kDa light (L) chain disulphide bonded to a 100 kDa heavy (H) chain (Helting et al, J. Biol. Chem., 252:187-193 (1977a); and Niemann et al, Molecular Biology of Clostridial Neurotoxins, Alouf Ed., Sourcebook of Bacterial Protein Toxins, Academic Press, London (1991)). The toxic activity of this protein lies within the L chain, a zinc-dependent protease (Schiavo

et al, EMBO J., 11:3577-3583 (1992)), which is thought to mediate the blockage of inhibitor release from neurons by proteolysis of synaptobrevin (Schiavo et al, Nature (London), 359:832-835 (1992)). The H chain is thought to initiate binding and uptake of the toxin at presynaptic membranes (Helting et al, J. Biol. Chem., 252:194-197 (1977b); and Morris et al, J. Biol. Chem., 255:6071-6076 (1980)). Digestion of the toxin molecule with papain yields a 50 kDa polypeptide, which corresponds to the C-terminal of the H-chain and a 100 kDa molecule corresponding to the L chain linked to the N-terminal of the H chain (Helting et al (1977a), supra). The 50 kDa polypeptide, termed TT fragment C (FC), is non-toxic but, possesses ganglioside (Halpern et al, Infect. Immun., 58:1004-1009 (1990); and Morris et al (1980), supra) and protein binding activities (Schiavo et al, FEBS. Lett., 290:227-230 (1991)). In early studies, vaccination of animals with FC derived by proteolysis of the native toxin was shown to protect them against subsequent lethal challenge with TT (Helting et al (1977a), supra). Furthermore, studies with monoclonal antibodies demonstrated that neutralizing epitopes exist within this molecule (Kenimer et al, Infect. Immun., 42:942-948 (1983)). Thus, FC was identified as a good candidate molecule for the production of an alternative TT vaccine.

Detailed Description Text (123):
7. Comparison of Serum IgG Anti-Salmonella LPS and S. typhi Flagella Induced by Intranasal Immunization with CVD 915 and CVD 908-htrA

Detailed Description Text (124):
One of the important issues to address during the evaluation of new candidate vaccine vectors is to compare the immune responses elicited by the new constructs (e.g., CVD 915) to that of the leading candidates (e.g., CVD 908-htrA) for which a large body of data is already available. With this objective, groups of 10 Balb/C mice were immunized intranasally with 10.sup.10 cfu of either attenuated strain CVD 915 or CVD 908-htrA, twice, 36 days apart. Mice were bled before immunization (day 0) and at days 35, 55 and 95 (CVD 915 only). Antibodies against LPS and flagella antigens were determined by ELISA as described by Tacket et al (1977), supra. Briefly, ELISA plates were coated with 5.0 .mu.g of each antigen, sera samples were tested in 8 2-fold dilutions, antibody titers were expressed as ELISA units/ml defined as the inverse of the dilution that produce 0.5 absorbance values at 492 nm. The results are shown in FIGS. 9 and 10.

Detailed Description Text (129):
In order to change the expression of the Vi antigen from osmotically regulated to constitutive, the promoter of vipR was focused upon (FIG. 1A). It is thought that the products of vipR, and ompR-envZ perform their regulatory action by binding the upstream region of vipR (Hashimoto et al (1996), supra). To this effect, it was postulated in the present invention that by substituting the promoter of vipR with a strong promoter, e.g., P.sub.tac, the down-regulation of vipR, and subsequently the control in the expression of the Vi antigen, would be eliminated. The promoter P.sub.tac is constitutive in Salmonella spp., as these organisms lack laqI. Accordingly, constitutive Vi antigen-expressor derivatives of CVD 915 and CVD 908-htrA were constructed in the following manner.

Detailed Description Text (133):
In parallel, other cassettes was constructed by insertion of sacB-neo between segment A and segment B referred to above. Initially, fragment A and fragment B were fused by PCR using both fragments as template and oligonucleotides SEQ ID NO: 7 and SEQ ID NO: 10 as primers, as described by Noriega et al (1996), supra). The resulting amplified fragment A-fragment B fusion was cloned in pGEM-T, using the pGEM-T Vector System kit, yielding pGEM-T::fragment A-fragment B. Then, sacB-neo was obtained by SmaI digestion of pIB729 (Blomfield et al, supra), and inserted in the SmaI site of pGEM-T::segment A-segment B. The resulting plasmid was named pGEM-T::vipR::sacB-neo. This plasmid was digested with SstI, effectively removing the vipR::sacB-neo allele, which was then cloned in the SstI site of the suicide vector pJG14, yielding pJG14::vipR::sacB-neo. pJG14 is a temperature-sensitive, pSC101 origin of replication-derived, chloramphenicol-selected, suicide plasmid (Galen et al, 96 th General Meeting, American Society for Microbiology, Abstract, page 529-H260 (1996)). In addition, the SstI-digested vipR::sacB-neo allele was cloned in the SstI site of suicide vector pKTN701 (Hone et al (1991), supra), yielding pKT::vipR::sacB-neo. S. typhi strain CVD 915 was electroporated with pJG14::vipR::sacB-neo. In parallel, S.

typhi strain CVD 908-htrA was electroporated with pKT::vipR::sacB-neo. Homologous recombination was carried out between pJG14::vipR::sacB-neo and the vipR gene in S. typhi CVD 915, using the procedures described by Noriega et al (1996), supra; and between pKT::vipR::sacB-neo, and the vipR gene in S. typhi CVD 908-htrA, using the procedures described by Hone et al (1991), supra, with the exception that double cross-over mutant selection was enhanced by isolating kanamycin-resistant, chloramphenicol-sensitive clones. The resulting S. typhi CVD 915-derivative and CVD 908-htrA-derivative strains did not express the Vi antigen due to the insertion/deletion in the vipR allele.

Detailed Description Text (134):
In the second phase, the constitutive P.sub.tac promoter was substituted for the sacB-neo insertion in the vipR locus of the S. typhi CVD 915-derivative and S. typhi CVD 908-htrA-derivative strains noted above (FIG. 1B). Specifically, the P.sub.tac -vipR segment in pBS::P.sub.tac -vipR was cloned into the BamHI-EcoRI site of pJG14, yielding pJG14::P.sub.tac -vipR. Plasmid pJG14::P.sub.tac -vipR was then used to exchange P.sub.tac for sacB-neo in the CVD 915- and CVD 908-htrA-derivative strains by homologous recombination, as described above. The isolation of double cross-over mutants was enhanced by the counter-selection provided by the toxicity to sucrose conferred by sacB, and reversion to kanamycin sensitivity. The resulting strain derived from CVD 915 was named CVD 916, which was deposited at the American Type Culture Collection on May 4, 1998, under ATCC No. 202116. The resulting strain derived from CVD 908--htrA was named CVD 909, which was deposited at the American Type Culture Collection on May 4, 1998, under ATCC No. 202117. Genotypically, the P.sub.tac insertion in both strains was characterized by PCR, demonstrating the insertion of P.sub.tac in the appropriate site.

Detailed Description Text (137):
As shown in Table 5 above, in the wild-type S. typhi strain Ty2 and the attenuated strains CVD 915 and CVD 908--htrA, the expression of the Vi antigen is highly dependent on the osmolarity (provided by the NaCl concentration) of the medium. In contrast, the expression of the Vi antigen in strains CVD 916 and CVD 909 is strong, constitutive, and not regulated by changes in osmolarity.

Detailed Description Paragraph Table (1):
TABLE 2 Invasion and Intracellular Growth in Henle 407 Cells Intracellular cfu.sup.a Strain Genotype 0 hr 4 hr 22 hr Ty2 wild-type 6.7 .times. 10.sup.3 3.1 .times. 10.sup.4 8.8 .times. 10.sup.4 CVD 915 .DELTA.guaB-A 3.3 .times. 10.sup.2 2.9 .times. 10.sup.2 2.5 .times. 10.sup.2 CVD 908 .DELTA.aroC, .DELTA.aroD 5.8 .times. 10.sup.2 1.3 .times. 10.sup.3 3.5 .times. 10.sup.3 CVD 908-htrA .DELTA.aroC, .DELTA.aroD, .DELTA.htrA 1.3 .times. 10.sup.3 3.4 .times. 10.sup.3 1.3 .times. 10.sup.3 .sup.a Colony forming units